

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

-89-

5299.

- 5 11. Copeland, N.G., Gilbert, D.J., Cho, B.C.,
Donovan, P.J., Jenkins, N.A., Cosman, D.,
Anderson, D., Lyman, S.D. and Williams, D.E.
(1990) Cell 63, 175-183.
- 10 12. Dexter, T.M. and Moore, M.A.S. (1977) Nature
269, 412-414.
13. Downing, J.R., Roussel, M.F. and Sherr, C.J.
(1989) Mol. Cell. Biol. 9, 2890.
- 15 14. Flanagan, J.G. and Leder, P. (1990). Cell 63,
185-194.
15. Flanagan, J.G., Chan, D. and Leder, P. (1991)
Cell 64, 1125-1135.
- 20 16. Fujita, J., Onoue, H., Ebi, Y., Nakayama, H.,
Kanakura, Y. and Kitamura, Y. (1989) Proc.
Natl. Acad. Sci. U.S.A. 86, 2888-2891.
- 25 17. Geissler, E.N., Ryan, M.A. and Housman, D.E.
(1988) Cell 55, 185-192.
18. Gluzman, Y. (1981) Cell 23, 175-182.
19. Gordon, M.Y. (1991) Cancer Cells 3, 127-133.
- 30 20. Kriegler, M. (1990) Gene transfer and expression:
A laboratory manual. (New York; Stockton Press)
21. Ladner, M.B., Martin, G.A., Noble, J.A.,

-91-

30. McCulloch, E.A., Siminovitch, L., Till, J.E.,
Russel, E.S., and Bernstein, S.E. (1965) *Blood*
26, 399-410.
- 5 31. McCulloch, E.A. (1970) In Regulation of
hematopoiesis, A.S. Gordon, ed. (New York:
Appleton), pp.649-675.
- 10 32. Mintz, B. and Russell, E.S. (1957) *J. Exp. Zool.*
134, 207-237.
33. Morrison-Graham, K. and Weston, J.A. (1989)
Trends Genet. 5, 116-121.
- 15 34. Naughton, M.A. and Sanger, F. (1961) *Biochem. J.*
78, 156-162.
- 20 35. Nocka, K., Majumder, S., Chabot, B., Ray, P.,
Cervone, M., Bernstein, A. and Besmer, P. (1989)
Genes & Dev. 3, 816-826.
- 25 36. Nocka, K., Tan, J., Chiu, E., Chu, T.Y., Ray P.,
Traktman, P. and Besmer, P. (1990a) *EMBO J.* 9,
1805-1813.
37. Nocka, K., Buck, J., Levi, E. and Besmer, P.
(1990b) *EMBO J.* 9, 3287-3294.
- 30 38. Nocka, K., Huang, E., Beier, D.R., Chu, T.Y.,
Buck, J., Lahm, H.W., Wellner, D., Leder, P. and
Besmer, P. (1990). *Cell* 63, 225-233.
39. Orr-Urtreger, A., Avivi, A., Zimmer, Y., Givol,
D., Yarden Y. and Lonaï, P. (1990) *Development*

-93-

Proc. Natl. Acad. Sci. USA 74, 5463-5467.

50. Sarvella, P.A. and Russel, L.B. (1956) J. Hered. 47, 123-128.
51. Silver, W.K. (1979) White-spotting, patch and rump-white. In the Coat Colors of Mice: A model for Gene Action and Interaction (New York: Springer-Verlag), pp. 206-241.
52. Stevens, L.C. (1979) Inbred Strains of mice. 11, 39.
53. Tan, J.C. Nocka, K., Ray, P., Traktman, P. and Besmer, P. (1990) Science 247, 209-212.
54. Todaro, G.J. and Green, H. (1963) J. Cell Biol. 17, 299-313.
55. Tushiniski, R.J., Oliver, I.T., Guilbert, L.J., Tynan, P.W., Warner, J.R. and Stanley, E.R. (1982) Cell 28, 71-81.
56. Williams, D.E., Eisenman, J., Baird, A., Rauch, C., Ness, K.V., March, C.J., Park, L.S., Martin, U., Mochizuki, D.Y., Bosell, H.S., Burgess, G.S., Cosman, D. and Stewart, D.L. (1990) Cell 63, 167-174.
57. Yarden, Y., Kuang, W.J., Yang-Feng, T., Coussens, L., Munemitsu, S., Dull, T.J., Chen, E., Schlessinger, J., Francke, U. and Ullrich, A. (1987) EMBO J. 6, 3341-3351.

-95-

67. Levi-Schaffer, F., Austen, K.F., Caulfield, J.P.,
Hein, A., Bloes, W.F. and Stevens, R.L. (1985) J.
Immunol. 135, 3454-3462.
- 5 68. Gregory, C.J. and Eaves, A.C. (1978) Blood 51,
527-537.
69. Iscove, N.N. (1978b). In Polastic Anemia, S.
Hibino, S. Takaku and N.T. Shahidi, eds. (Tokyo:
10 University of Tokyo Press), pp. 31-36.
70. Das, S.K. and Stanley, E.R. (198) J. Biol.
Chem. 257, 13679.
- 15 71. Gough, N.M. and Williams, L.R. (1989) Cancer
Cells 1, 77-80.
72. Kitamura, Y., Go, S., and Hatnaka, K. (1978).
Blood 52, 447-452.
- 20 73. Kitamura, Y., and Fujita, J. (1989). Blood 53,
492-497.
74. Nakahata, T., Kobayashi, T., Ishiguro, A., Tsuji,
25 K., Naganuma, K., Ando, O., Yagi, Y., Tadokoro,
K. and Akabane, T. (1986) Nature 324, 65-67.
75. Tsuji, K., Natakata, T., Takagi, M., Kobayashi,
30 T., Ishiguro, A., Kikuchi, T., Naganuma, K.,
Koiki, K., Miyajima, A., Arai, K., Akabane,
T. (1990a) J. Immunol. 144, 678-684.
76. Tsuji, K., Nakahata, T., Takagi, M., Kobayashi,

-97-

142, 2405-2417.

- 5 85. Schmidt, E.V., Paterngale, P.K., Weir, L. and Leder, P. (1988). Proc. Natl, Acad. Sci. USA 85, 6047-6051.
- 10 86. Lehrach, H., Diamond, D., Wozney, J.M., and Boedite, H. (1977). RNA molecular weight determinations by gel electrophoresis under denaturing conditions-a critical reexamination. Biochemistry 16, 4743.
- 15 87. Feinberg, A.P., and Vogeistein, B. (1963). Anal. Biochem. 132, 6-13.
88. Stanley, E.R., and Guilbert, L.J. (1961). J. Immunol. Meth. 42, 263-264.
- 20 89. Scherr, C.J., Rettenmier, C.W., Sacca, R., Roussel, M.F., Look, A.T. and Stanley, E.R. (1965). Cell 41, 666-676.
- 25 90. Chui, D.K., Liato, S.K., and Walker, K. (1978). Blood 51, 539-547.
91. Avner, P., Amar. L., Dandolo, L., and Guenet, J.L. (1968). Trends Gene, 4, 18-23.
- 30 92. Yung, et al. (1981) J. Immunol. 127, 794-799.
93. Stevens, R.L. and Austen, K.F. (1989), Immunol. Today 10, 381-386.
94. Schrader, J.W. (1981) J. Immunol. 126, 452-460.

-99-

What is claimed is:

- 5 1. A purified mammalian protein corresponding to a c-~~kit~~ ligand which comprises a homodimer of two polypeptides, each polypeptide having a molecular weight of about 30 kilodaltons and an isoelectric point of 3.8.
- 10 2. A purified mammalian protein of claim 1, wherein the mammalian protein is a murine protein.
3. A purified mammalian protein of claim 1, wherein the mammalian protein is a human protein.
- 15 4. A purified mammalian protein corresponding to a c-~~kit~~ ligand which comprises a homodimer of two polypeptides, each polypeptide having a molecular weight of about 30 kilodaltons, an isoelectric point of 3.8, and wherein the two polypeptides
20 are linked by a disulfide bond.
- 25 5. A pharmaceutical composition comprising the purified mammalian protein of claims 1 or 4 and a pharmaceutically acceptable carrier.
- 30 6. A pharmaceutical composition for the treatment of leucopenia in a mammal, which comprises an effective amount of the pharmaceutical composition of claim 5 and an effective amount of a factor selected from the group consisting of G-CSF, GM-CSF and IL-3, effective to treat leucopenia in a mammal.
7. A pharmaceutical composition for the treatment of

-101-

symptoms of defective lung development which comprises an effective amount of the composition of claim 5, effective to treat infants exhibiting symptoms of defective lung development.

5

13. A composition for the prevention of hair loss in a subject which comprises an effective amount of the pharmaceutical composition of claim 5, effective to prevent hair loss in the subject.

10

14. A composition for inhibiting the loss of pigment in a subject's hair, which comprises an effective amount of the pharmaceutical composition of claim 5, effective to prevent the loss of pigment in the subject's hair.

15

15. An isolated nucleic acid molecule which encodes an amino acid sequence corresponding to a c-kit ligand (KL).

20

16. An isolated nucleic acid molecule of claim 15, wherein the c-kit ligand (KL) is a human c-kit ligand (KL).

25

17. An isolated nucleic acid molecule of claim 15, wherein the c-kit ligand (KL) is a murine c-kit ligand (KL).

30

18. An isolated nucleic acid molecule of claim 15, wherein the nucleic acid molecule is a DNA molecule.

19. The DNA molecule of claim 18, wherein the DNA molecule is a cDNA molecule.

-103-

- 5
30. A vector of claim 28 which comprises a virus.
31. A host vector system for the production of an amino acid sequence which is the c-kit ligand which comprises the plasmid of claim 29 in a suitable host.
- 10
32. A host vector system of claim 31, wherein the suitable host is a eucaryotic cell.
33. A host vector system of claim 32, wherein the eucaryotic cell is a mammalian cell.
- 15
34. A host vector system of claim 32, wherein the eucaryotic cell is an insect cell.
35. A host vector system of claim 32, wherein the eucaryotic cell is a yeast cell.
- 20
36. A host vector system of claim 31, wherein the suitable host is a procaryotic cell.
- 25
37. A c-kit ligand (KL) polypeptide wherein the c-kit ligand (KL) polypeptide comprises a fragment of the protein of claim 1.
- 30
38. A mutated c-kit ligand (KL) polypeptide wherein the biological activity mediated by the binding of the ligand to the receptor is destroyed.
39. A substance capable of specifically forming a complex with the c-kit ligand (KL) polypeptide of claim 1 or 37.

-105-

pharmaceutically acceptable carrier.

- 5 49. A method of modifying a biological function associated with c-kit cellular activity which comprises contacting a cell, whose function is to be modified, with an effective amount of the pharmaceutical composition of claim 5, effective to modify the biological function of the cell.
- 10 50. The method of claim 49, wherein the biological function is the propagation of a cell that expresses c-kit.
- 15 51. The method of claim 50, wherein the cell which expresses c-kit is a hematopoietic cell.
52. The method of claim 49, wherein the biological function is in vitro fertilization.
- 20 53. A method of modifying a biological function associated with c-kit cellular activity in a patient which comprises administering to the patient an effective amount of the pharmaceutical composition of claim 5, effective to modify the biological function associated with c-kit function.
- 25 54. A method of stimulating the proliferation of mast cells in a patient which comprises administering to the patient an effective amount of the pharmaceutical composition of claim 5, effective to stimulate the proliferation of the mast cells in the patient.
- 30

-107-

- 5 61. A method of treating allergies in a patient which comprises administering to the patient an effective amount of the pharmaceutical composition of claim 5, effective to treat the allergy.
- 10 62. A method of treating melanoma in a patient, which comprises administering to the patient an effective amount of the composition of claim 5, effective to treat the melanoma.
- 15 63. A method for the treatment of leucopenia in a patient which comprises administering an effective amount of the composition of claim 6.
- 20 64. A method for the treatment of anemia in a patient which comprises administering an effective amount of the composition of claim 7.
- 25 65. A method for enhancing engraftment of bone marrow during transplantation in a patient which comprises administering an effective amount of the composition of claim 8.
- 30 66. A method of enhancing bone marrow recovery in treatment of radiation, chemical, or chemotherapeutic induced bone marrow aplasia or myelosuppression which comprises treating patients with therapeutic effective doses of the composition of claim 9.
67. A method of treating acquired immune deficiency in a patient which comprises administering to the patient a therapeutically effective amount of the

-109-

- 5
73. A method of treating infants exhibiting symptoms of defective lung development which comprises administering to the infant a therapeutically effective amount of the polypeptide of claim 12.
- 10
74. A method of preventing the loss of hair in a patient which comprises administering to the patient an effective amount of the composition of claim 13.
- 15
75. A method of inhibiting the loss of pigment in a subject's hair which comprises administering to the subject an effective amount of the composition of claim 14.
- 20
76. A method for measuring the biological activity of a c-kit (KL) polypeptide which comprises:
- 25
- a) incubating normal bone-marrow mast cells with a sample of the c-kit ligand (KL) polypeptide under suitable conditions such that the proliferation of the normal bone-marrow mast cells are induced;
- 30
- b) incubating doubly mutant bone-marrow mast cells with a sample of the c-kit ligand (KL) polypeptide under suitable conditions;
- c) incubating a. and b. with ^3H -thymidine;
- d) determining the amount of thymidine incorporated into the DNA of the normal bone-marrow mast cells and the doubly mutant bone-marrow mast cells; and

1/55

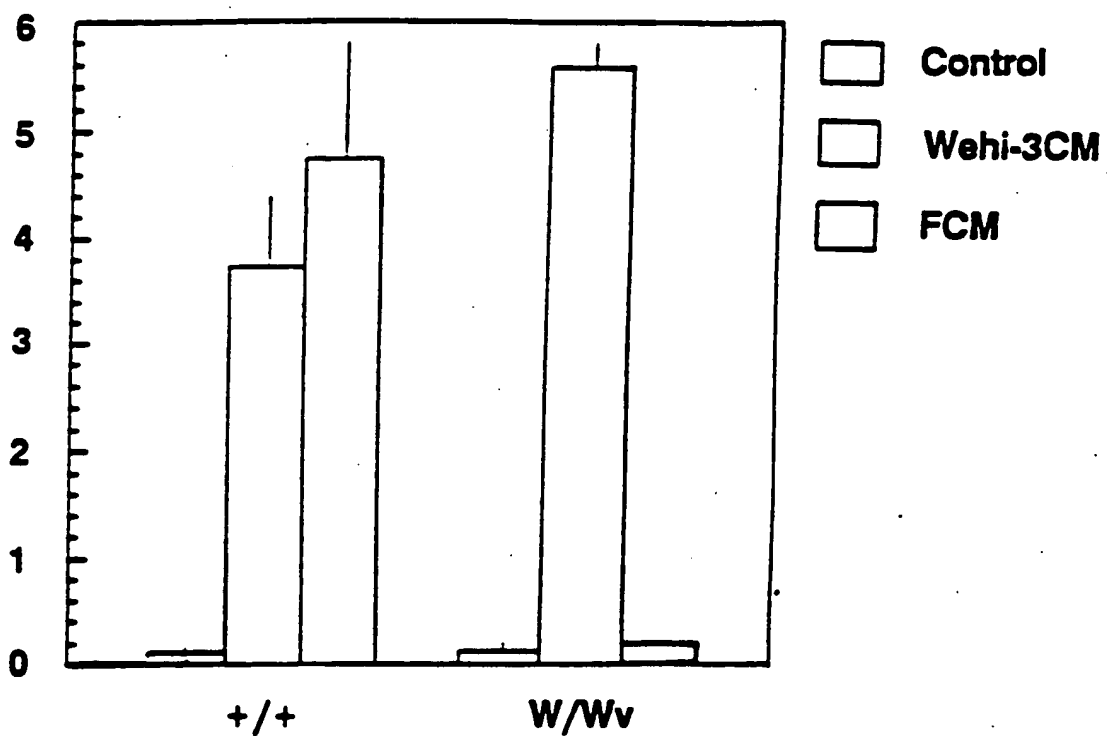
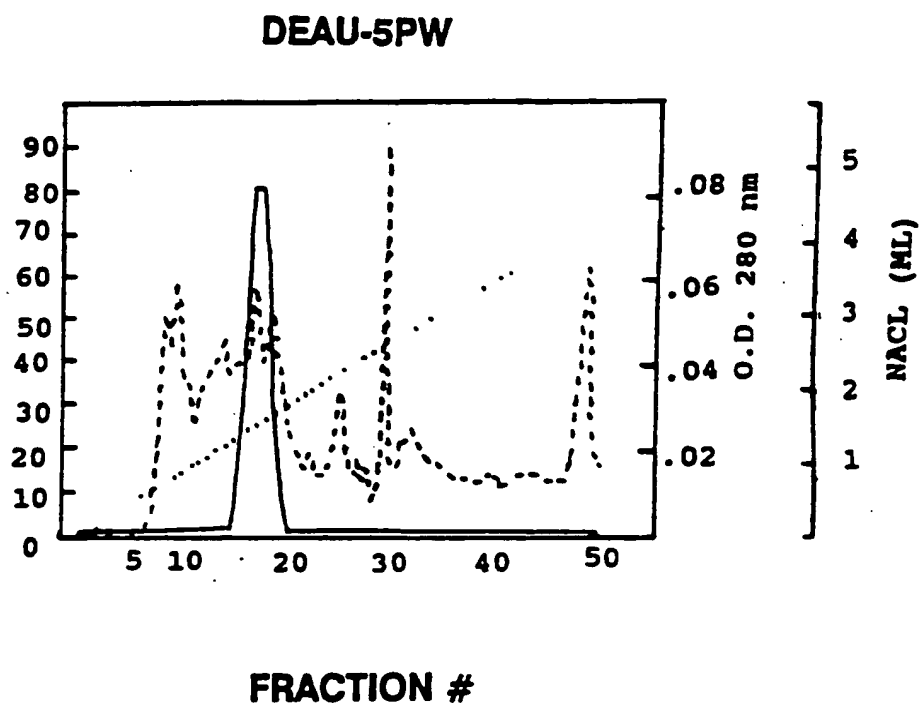
CPM X 10⁻³

FIGURE 1

SUBSTITUTE SHEET

3/35

FIGURE 2 B



5/35

FIGURE 2 D

ANALYTICAL C 18

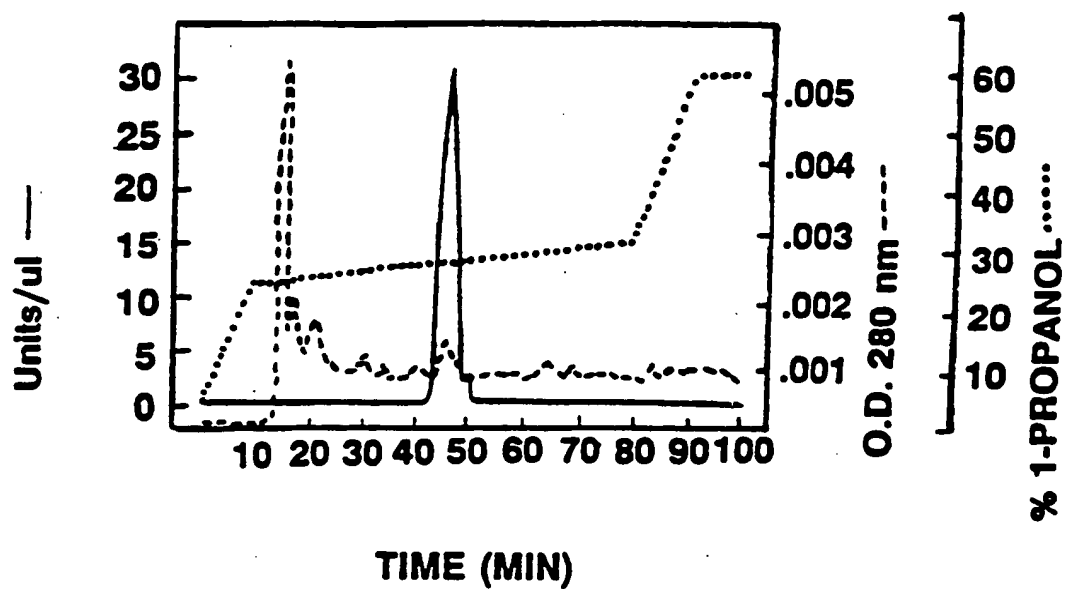
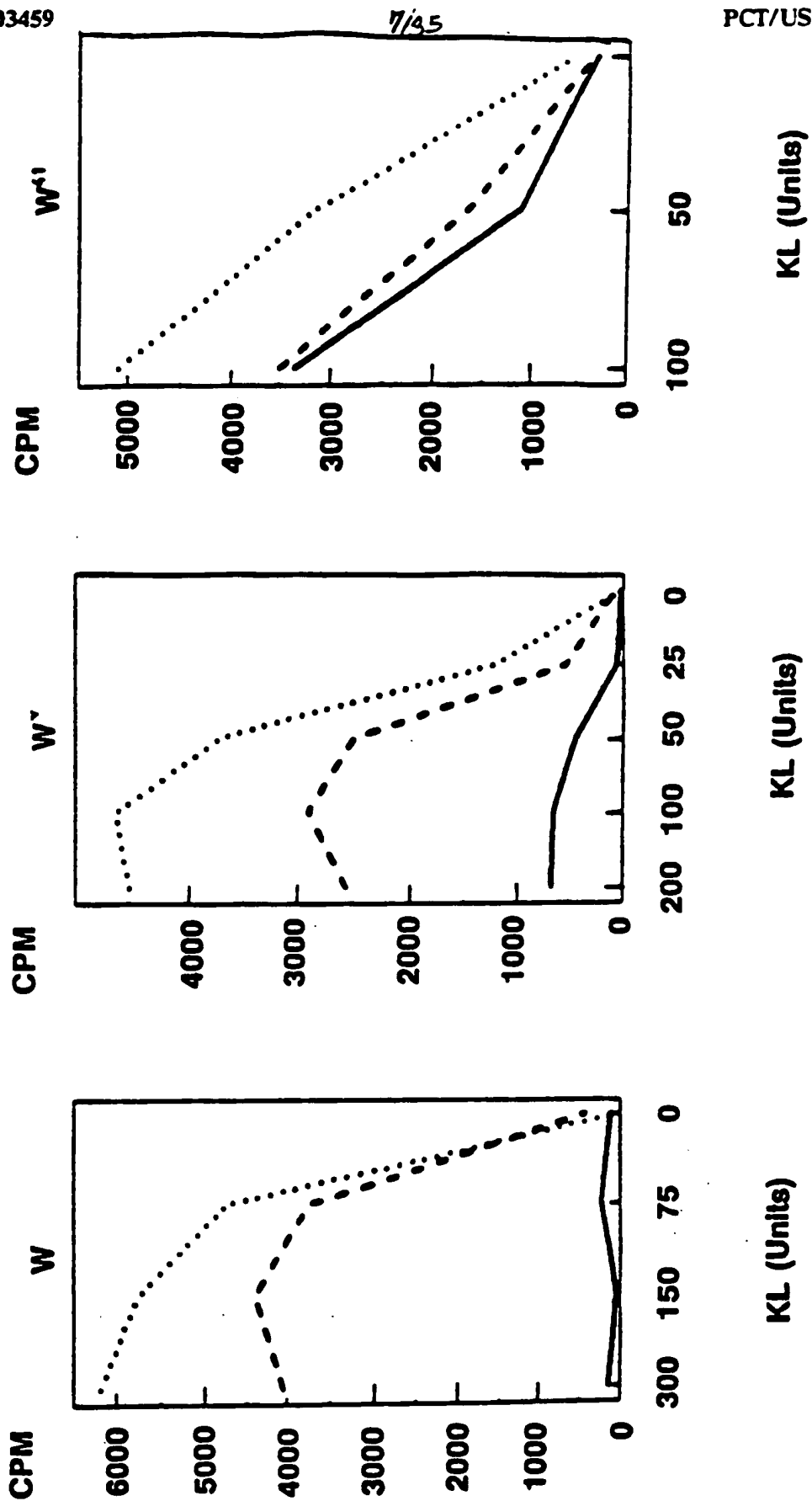


FIGURE 4



9/35

C-KIT SURFACE EXPRESSION

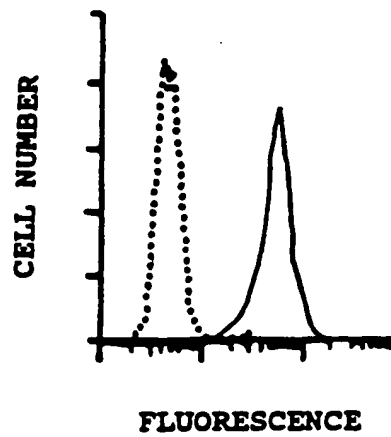
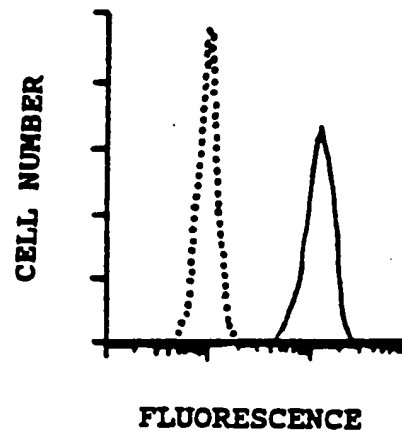
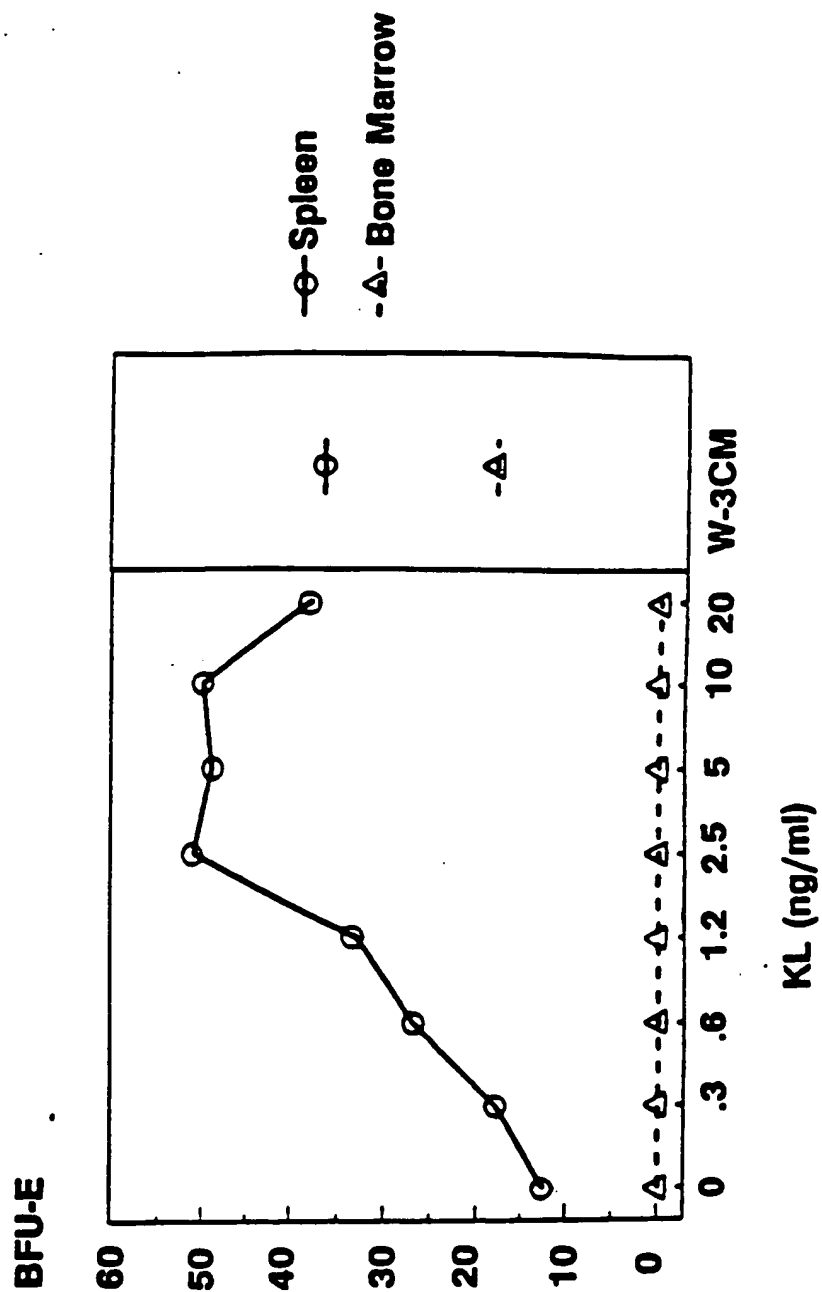


FIGURE 5 B

11/35

FIGURE 6



10 20 30
KEIXGNPVT D N V K D I T K L V A N L P N O Y N I T L N Y V A G N XVL P

5' C G T C 3'
aagcttgataatgttaaagacattacaaaactggtggcaaatcttccaaatgactatatgatgataccctcaattacgtggccggaatgggatcc

cgccaagcttgataatgttaaagatatattac 3'
5' C C G G C C A
T C
3' TTAATACAGCGGCCGTACccctaggggcc 5'
G G T T T
C C C
A A A

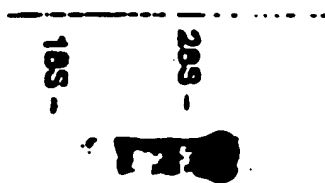
FIGURE 8

15/35



FIGURE 10

17/35



190

FIGURE 12

19/35

FIGURE 14

Figure 14 A

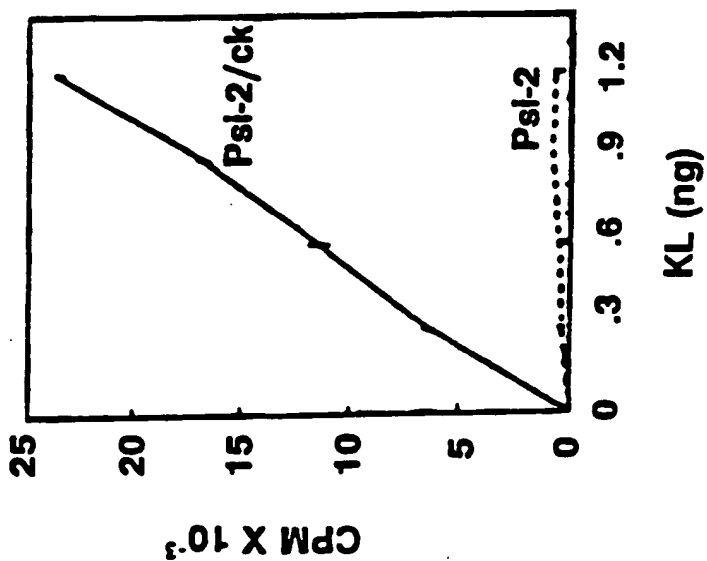
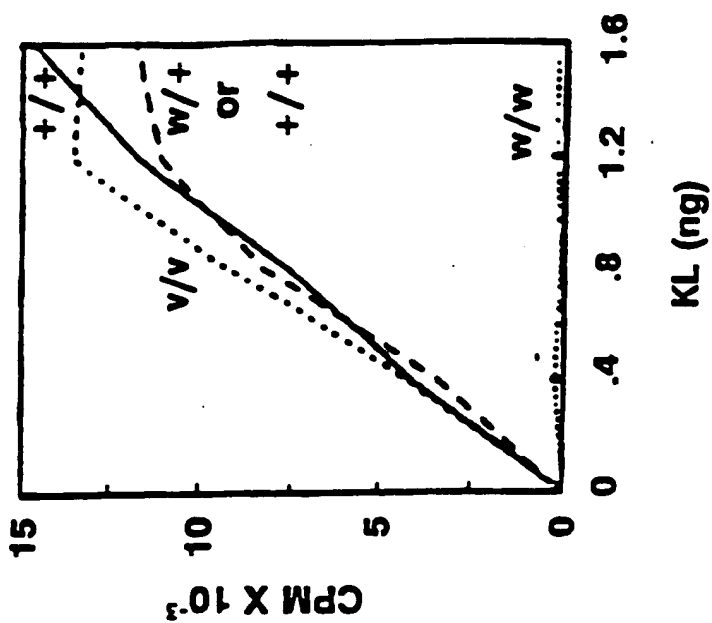


Figure 14B



21/35

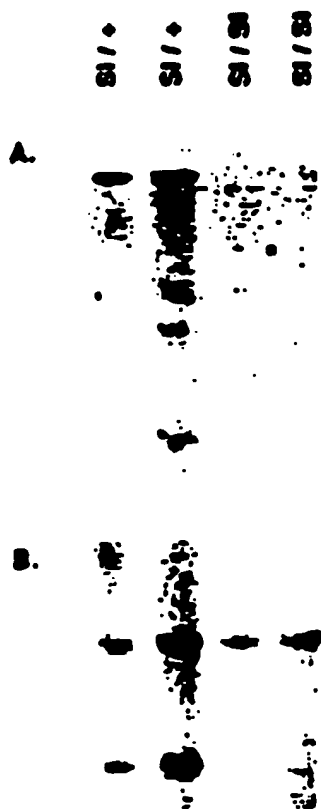


FIGURE 16

29/35



FIGURE 18A

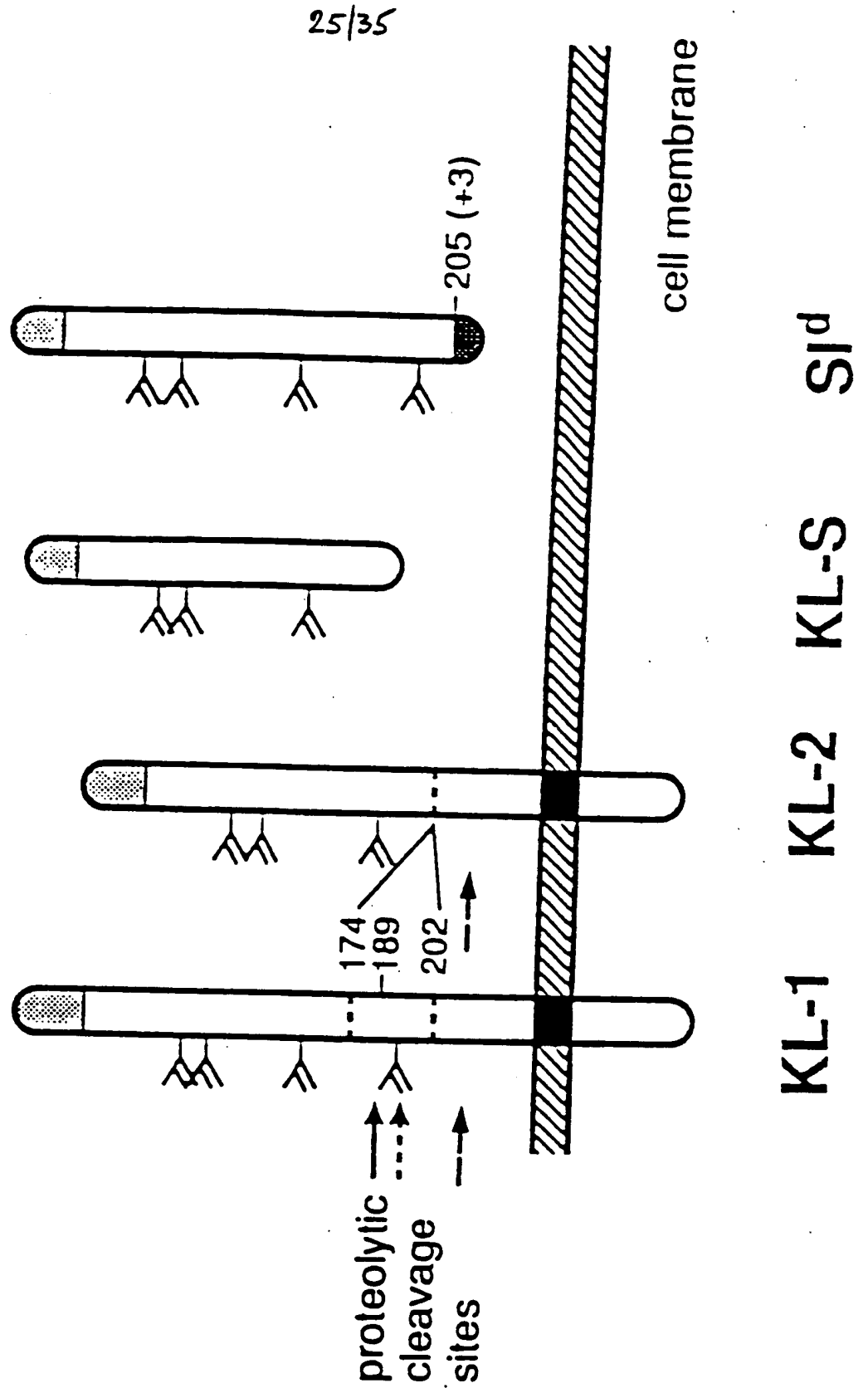


FIGURE 19



FIGURE 21A

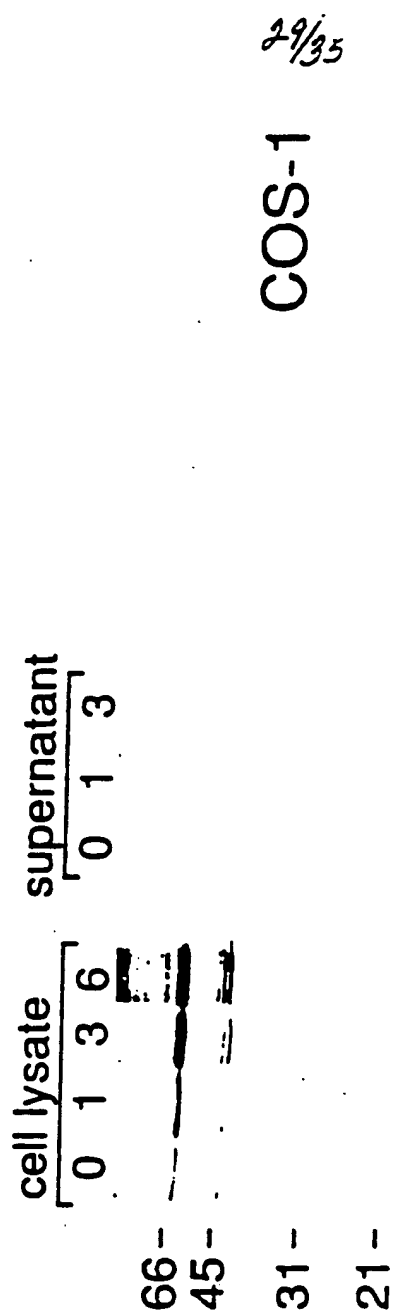


FIGURE 21C

3/35

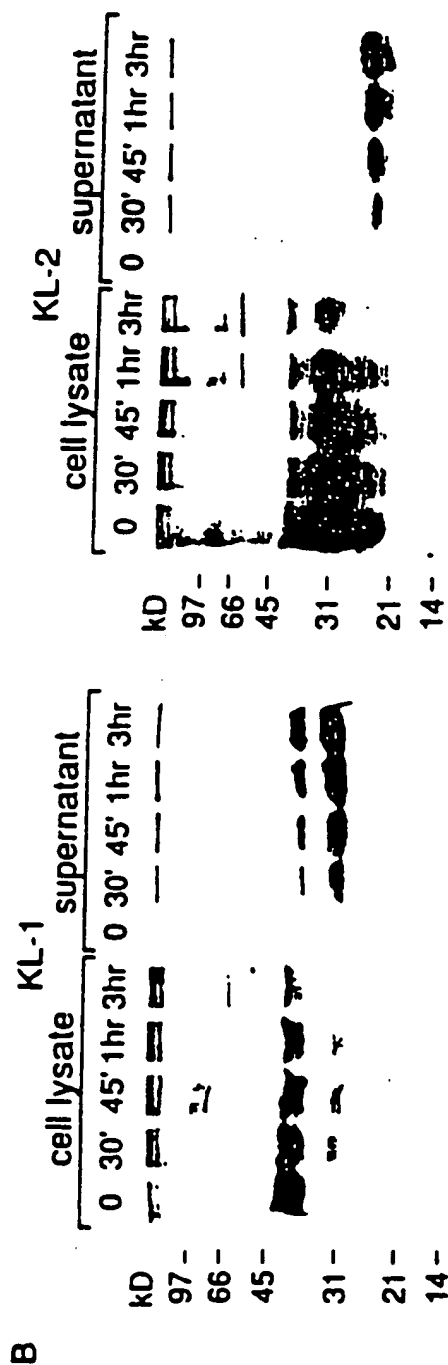


FIGURE 22B

33/35

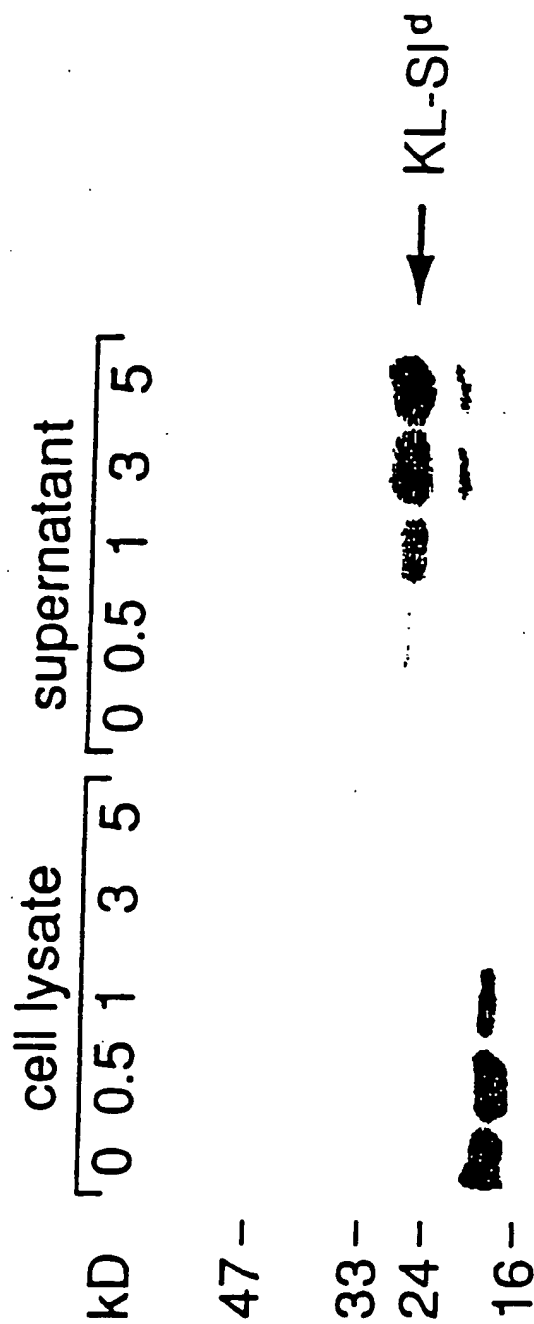


FIGURE 23A

35/35

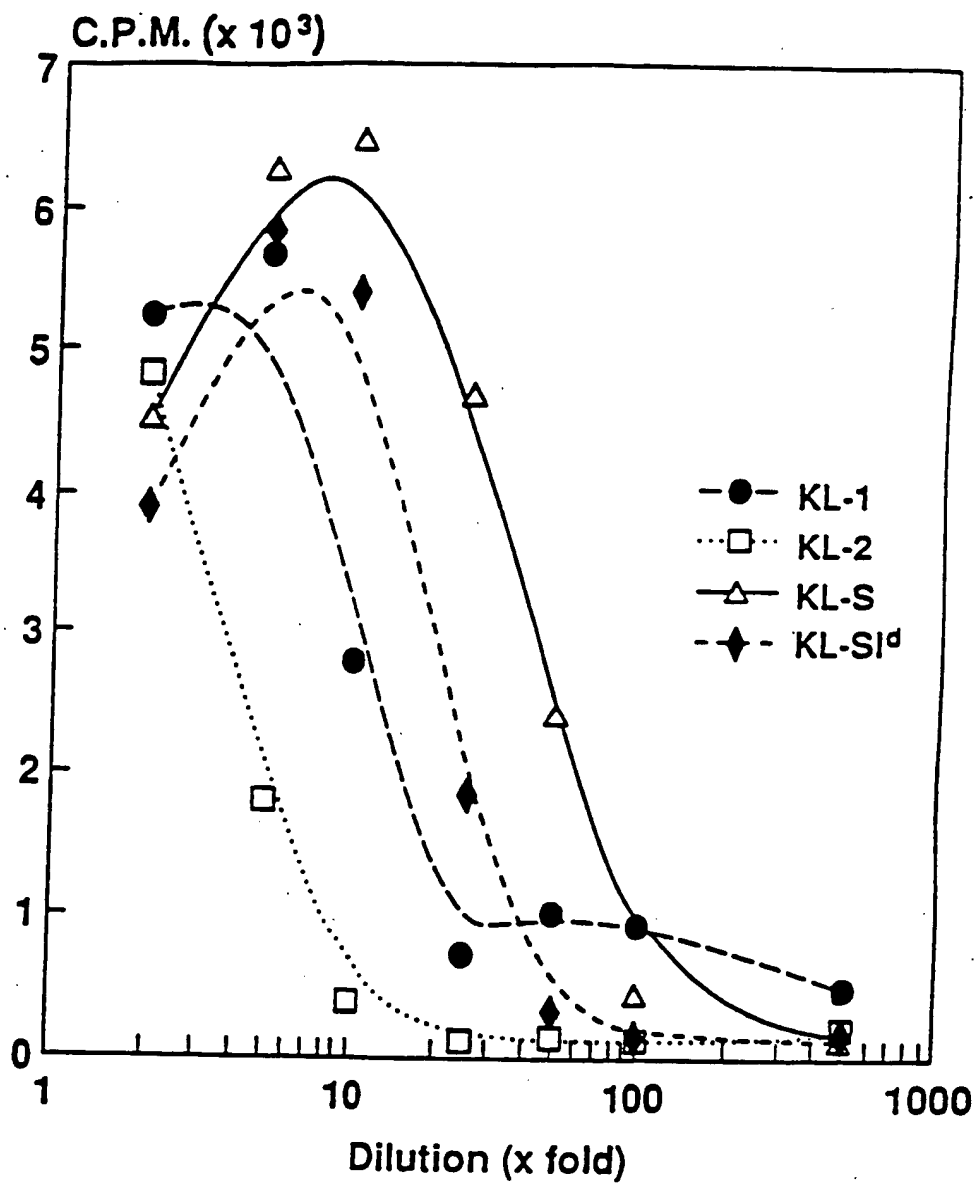


FIGURE 24

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter ¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹³, specifically:

3. ☐ Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this international application as follows:

See attachment

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
1-5, 11-37, 46-47, 49-53
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.